**(For scientific research use only, not for clinical diagnosis!)**

# Rabbit Apolipoprotein B100 (apo-B100)

## ELISA Kit Instructions for Use Product No.:

## BY-ER772444 Specifications: 48T/96T

## Detection Range: 62.5 ng/mL– 2000 ng/mL.

**Sensitivity: The lowest detectable dose is less than 10 ng/mL.**

**Precision: intra-batch variation coefficient CV% is less than 10%; inter-batch variation coefficient CV% is less than 15%.**

**Recovery rate: The recovery rate is between 85%-115%.**

**Specificity: This kit recognizes native and recombinant rabbit apolipoprotein B100 (apo-B100) and has no crossover with structural analogs. Stability: Stored at 2℃-8℃, validity period is 6 months.**

**Purpose: Used to detect the concentration of rabbit apolipoprotein B100 (apo-B100) in samples such as serum, plasma, cell culture supernatant and tissue.**

**Please read the instructions carefully before use. If you have any questions, please contact us through the following methods: Official hotline: 025-5229-8998 Sales department phone: 13914481711 Technical phone: 15950492658 Company website: www.byabscience.cn For the specific shelf life, please refer to the outer packaging label of the kit. Please use the kit within the shelf life.**

When contacting us, please provide the product number and production date (see box label) so that we can serve you more efficiently.

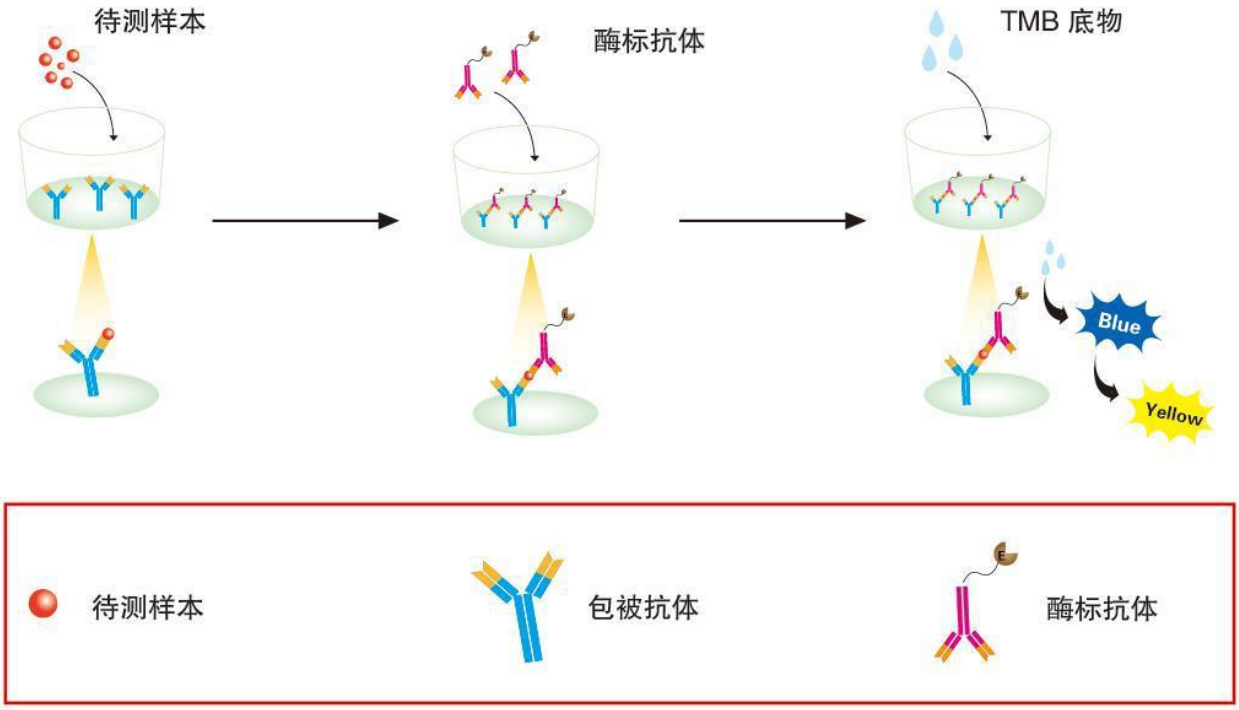
**Nanjing BYabscience technology Co.,Ltd**

**Website: www.byabscience.cn** **Official hotline: 025-5229-8998** **Supervision phone number:**

## Experimental principle

This kit uses double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). In the microwell enzyme plate pre-coated with anti-rabbit apolipoprotein B100 (apo-B100) antibody (solid-phase antibody), add rabbit apolipoprotein B100 (apo-B100) calibrator and sample to be tested, and then add HRP Labeled anti-rabbit apolipoprotein B100 (apo-B100) antibody (enzyme-labeled antibody), after incubation and sufficient washing, removes unbound components, and forms a solid-phase antibody-antigen-enzyme label on the solid surface of the microplate. Antibody sandwich complexes. Adding substrates A and B, the substrates are catalyzed by HRP to produce a blue product, which is finally converted to yellow under the action of the stop solution (acidic solution). The absorbance (OD value) is measured at a wavelength of 450 nm on a microplate reader. The absorbance (OD value) is positively correlated with the concentration of rabbit apolipoprotein B100 (apo-B100) in the sample to be tested. By fitting the calibrator curve, the concentration of rabbit apolipoprotein B100 (apo-B100) in the sample can be calculated.

## Experimental schematic diagram




**Nanjing BYabscience technology Co.,Ltd**

**Kit components and storage Unopened kits should be stored at 2-8 degrees Celsius. Do not use expired kits.**

| Components | **48-well configuration** | **96-well configuration** | Store after opening |
|---|---|---|---|
| Pre-coated enzyme plate | 48T | 96T | **2-8℃14 days** |
| Standard product | 0.3mL*6 tubes | 0.3mL*6 tubes | **2-8℃14 days** |
| sample diluent | 3ml | 6ml | 2-8℃180 days |
| HRP labeled antibodies | 5ml | 10ml | **2-8℃14 days** |
| Chromogenic substrate A | 3ml | 6ml | 2-8℃180 days |
| Chromogenic substrate B | 3ml | 6ml | 2-8℃180 days |
| stop solution | 3ml | 6ml | 2-8℃180 days |
| **20×Lotion** | 15ml | 25ml | 2-8℃180 days |
| sealing film | 2 sheets | 2 sheets | |
| manual | 1 serving | 1 serving | |
| Ziplock bag | 1 | 1 | |

The concentrations of calibrators are: 2000, 1000, 500, 250, 125, 62.5 ng/mL.

Note: 1: Before use, please check whether the label and quantity of the reagents in the kit are consistent with the table.

2: If the components of the kit need to be used again, please ensure that they have not been contaminated since the last use. 3: If the enzyme plate is not used up in a single time, remember to seal it and store it at 2-8°C.

**Prepare your own test equipment required for the test (not provided, but can assist in**

1) Microplate reader capable of detecting absorbance at 450 nm 2) Pipette, pipette tip, and sample addition tank 3) 37°C incubator or water bath 4) Test tubes, centrifuge tubes, measuring cylinders, etc. for preparing

reagents 5) Distilled water or deionized

water Ionized water

6) Vortex shaker, microplate shaker

**Notes 1) For scientific research use only, not for clinical diagnosis.**

2) Use within the validity period marked on the kit. Expired products must not be used.

3) Do not mix with kits or components from other manufacturers. Use the sample diluent provided with the kit.

4) If the sample value is higher than the highest standard concentration value, please dilute the sample appropriately and then re-measure.

5) Human anti-mouse and other heterophilic antibodies present in the sample to be tested will interfere with the test results. Please eliminate this factor before testing.

6) The test results obtained by other methods are not directly comparable to the test results of this kit.

7) Please wear a lab coat and latex gloves for protection during the test. Especially when testing blood or other body fluid samples, please follow the national biological laboratory safety protection regulations.

8) Carry out incubation strictly according to the specified time and temperature to ensure accurate results. All reagents must reach room temperature 20-25°C before use. Store reagents refrigerated immediately after use.

9) Improper plate washing can lead to inaccurate results. Make sure to absorb as much liquid as possible from the wells before adding substrate. Do not allow the microwells to dry out during incubation.

10) Eliminate residual liquid and fingerprints on the bottom of the plate, otherwise it will affect the OD value.

11) The substrate chromogenic solution should be colorless or very light in color.

12) Avoid cross-contamination of reagents and specimens to avoid erroneous results.

13) Avoid direct exposure to strong light during storage and incubation.

14) The microplate reader used for detection needs to be equipped with a filter capable of detecting a wavelength of 450±10nm, and the optical density range is between 0-3.5. It is recommended to preheat 15 minutes in advance before use.

15) The EP tubes and suction tips used in the test are single-use and are strictly prohibited from mixing.

**Nanjing BYabscience technology Co.,Ltd**

**Website: www.byabscience.cn** **Official hotline: 025-5229-8998** **Supervision phone number:**

## Sample preparation and storage

The following lists only general guidelines for sample collection and preservation. During the collection and storage of all samples, sodium azide shall not be used as a preservative. If the sample is not analyzed immediately, it should be aliquoted and stored frozen, and repeated freezing and thawing should be avoided.

**Cell culture supernatant - centrifuge to remove precipitate, analyze immediately or aliquot and store frozen at -20°C.**

**Serum - Collect blood in a clean test tube, coagulate at room temperature for 30 minutes, centrifuge at 2000×g for 20 minutes, and collect serum. Analyze immediately or aliquot and store frozen at -20°C.**

**Plasma—anticoagulate with heparin, citrate, or EDTA, and centrifuge at 2000×g for 20 minutes at 2-8°C within 30 minutes of blood draw. To eliminate the influence of platelets, it is recommended to further centrifuge at 10,000 × g for 10 minutes at 2-8°C. Analyze immediately or aliquot and store frozen at -20°C.**

**Cell lysis buffer - For adherent cells, remove the culture medium and wash with PBS, normal saline or serum-free culture medium. Add an appropriate amount of lysis solution and pipet several times with a gun to fully contact the lysate and cells. Typically after 10 seconds, cells are lysed. For suspended cells, collect the cells by centrifugation and wash them with PBS, physiological saline or serum-free culture medium. Add an appropriate amount of lysis solution, blow the cells with a gun, and flick them with your fingers to fully lyse the cells. After full lysis, centrifuge at 10000-14000×g for 3-5 minutes and take the supernatant. Analyze immediately or aliquot and store frozen at -20°C.**

**组织匀浆——用预冷的 PBS (0.01M, pH=7.4)冲洗组织，去除残留血液（匀浆中裂解的红细胞会影响测量结果），称重后将组织剪碎。将剪碎的组织与对应体积的 PBS（一般按 1:9 的重量体积比，比如 1g 的组织样品对应 9mL 的 PBS，具体体积可根据实验需要适当调整，并做好记**

录。推荐在 PBS 中加入蛋白酶抑制剂）加入玻璃匀浆器中，于冰上充分研磨。为了进一步裂解 组织细胞，可以对匀浆液进行超声破碎，或反复冻融。最后将匀浆液于 5000×g 离心 5~10 分 钟，取上清检测。

尿液——用无菌管收集，离心 2000×g 20 分钟。仔细收集上清。如有沉淀形成，应再次离心。

**Nanjing BYabscience technology Co.,Ltd**

网址：www.byabscience.cn　　官方热线：025-5229-8998　　监督电话：15950492658

**试剂准备 1、使用前，所有的组分都要至少复温 60min，确保充分复温到室温。**

2、浓缩洗涤液：从冰箱取出的浓缩洗涤液，会有结晶产生，这属于正常现象，水浴加热使结晶 完全溶解。浓缩洗涤液与蒸馏水，按 1:20 稀释，即 1 份的浓缩洗涤液，添加 19 份的蒸馏水。3、底物：底物液 A 和 B，在使用前，按 1:1 体积充分混合，混合后 15 分钟内使用。

操作程序 所有试剂和组分都先恢复到室温，标准品、质控品和样品，建议做复孔。

1、按前面说明书描述的方法，配制好试剂盒各种组分的工作液。

2、从铝箔袋中取出所需板条，剩余的板条用自封袋密封放回冰箱。

3、设置标准品孔、0 值孔、空白孔和样本孔，标准品孔各加不同浓度的标准品 50μL，0 值孔加样本稀释液 50μL，空白孔不加，样本孔加待测样本 50μL。

4、除空白孔外，标准品孔、0 值孔和样本孔，加入辣根过氧化物酶（HRP）标记的检测抗体 100 μL。

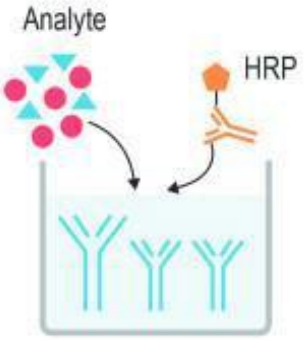
5、用封板膜盖住反应板，37℃水浴锅或恒温箱避光孵育 60min。

6、揭开封板膜，弃去液体，吸水纸上拍干，每孔加满洗涤液，静置 20S，甩去洗涤液，吸水纸上拍干，如此重复 5 次。若使用自动洗板机，请按洗板机操作程序进行洗板，添加浸泡 30s 的程序，可以提高检测的精度。洗板结束，加底物前，要在干净不掉屑的纸上，充分拍干反应板。（提示：为获得理想的实验结果，必须彻底移除残留液体。洗板完成之后，请立即进行下步操 作，不要让微孔板干燥。）7、将底物 A 和 B 按 1:1 体积充分混合，所有孔中加入底物混合液 100μL。用封板膜盖住反应 板，37℃水浴锅或恒温箱避光孵育 15min。

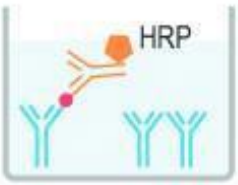
8、所有孔加入终止液 50μL，在 450nm 波长酶标仪上读取各孔吸光度（OD 值）。

网址：www.byabscience.cn 官方热线：025-5229-8998 监督电话：15950492658

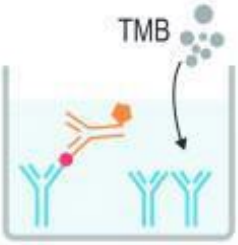
[操作流程图]



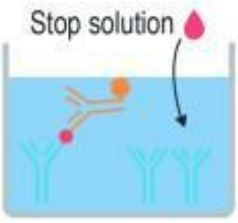

1. 对应板孔中加入50μL标准品工作液或样本后，立即每孔加入100ulHRP酶标抗体工作液，37°C孵育60分钟




2. 弃掉板内液体，洗板5次




3. 每孔加入底物A溶液50ul，底物B溶液50ul

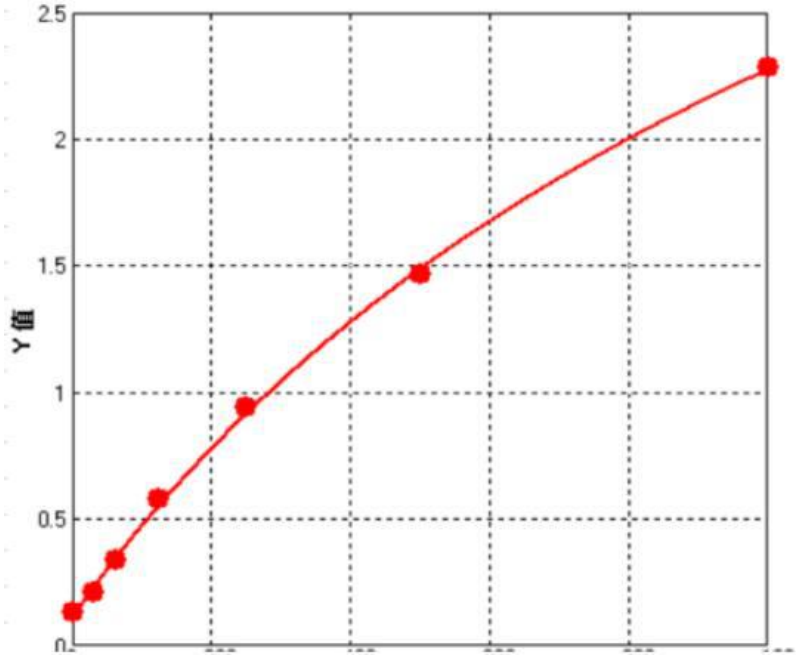



4. 每孔加入50μL终止液

5. 立即在450nm波长下读数，处理数据

## 结果计算

9、以标准品浓度做为横坐标，对应的吸光度（OD 值）作为纵坐标，利用计算机软件，采用四参数 Logistic 曲线拟合（4-pl），创建标准曲线方程，通过样本的吸光度（OD 值），利用方 程计算样品的浓度值。【用 ELISA Calc 软件计算，标曲建议使用四参数拟合，但不是唯一拟合方式】10、如果样品被稀释，通过上述方法测的的浓度值，要乘以稀释倍数，才是样品的最终浓度。注意：实验者需根据自己的实验建立标准曲线。每次检测，每块酶标板都必须设立标准曲线。以下曲线仅供参考！




（标曲示意图，仅供参考）

[问题分析] 若实验效果不好，请及时对显色结果拍照，保存实验数据，保留所用板条及 未使用试剂，然后联系我公司技术支持为您解决问题。同时您也可以参考以下资料：[问题解答]

| 问题描述 | 可能原因 | 相应对策相应对策 |
|---|---|---|
| 标准曲线梯度差 | 吸液或加液不准 | 检查移液器及吸头 |
| | Equilibration time is too short | Ensure sufficient balancing time |
| | Incomplete washing | Ensure the washing time and number of washes and the amount of liquid added to each hole |
| Very weak or colorless | Incubation time too short | Ensure adequate incubation time |
| | The experimental temperature is incorrect | Use recommended experimental temperatures |
| | Insufficient reagent volume or missing addition | Check the liquid aspirating and adding process to ensure that all reagents are added in order and in |
| | Incorrect dilution | |
| | Enzyme label inactivation or substrate failure | Mix enzyme conjugate and substrate and check by rapid color development |
| Reading value is low | Microplate reader settings are incorrect | Check the wavelength and filter |
| | | Turn on the microplate reader and preheat it in advance |
| Large coefficient of variation | Adding fluid incorrectly | Check the filling situation |
| High background value | The working concentration of the | Use the recommended dilution |
| | Incomplete washing of enzyme plate | Ensure that each step of cleaning is complete; if using an automatic plate washer, please check whether all outlets are blocked; |
| | The lotion is contaminated | Prepare fresh lotion |
| Low sensitivity | Improper storage of ELISA kits | Store relevant reagents according to |
| | Not terminated before reading | Stop solution should be added to |

## statement

1. Due to the current conditions and scientific and technological level, it is not possible to conduct comprehensive identification and analysis of all raw materials. This product may have certain quality and technical risks.

2. This kit removes/reduces some endogenous interfering factors in biological samples during the development process. Not all possible influencing factors have been removed.

3. The final experimental results are closely related to factors such as the effectiveness of the reagents, the relevant operations of the experimenter, and the experimental environment at the time. Our company is only responsible for the kit itself and is not responsible for the sample consumption caused by the use of the kit. Please use The user should fully consider the possible usage of the sample and reserve sufficient samples before use.

4. In order to achieve good experimental results, please only use the reagents provided in our company's kits, do not mix products from other manufacturers, and operate in strict accordance with the instructions.

5. Due to incorrect reagent preparation and microplate reader parameter settings during the operation, abnormal results may result. Please read the instructions carefully and adjust the instrument before the experiment.

6. Even if operated by the same personnel, different results may be obtained in two independent experiments. In order to ensure the reproducibility of the results, it is necessary to control every step of the experimental process.

7. The kits will undergo strict quality inspection before shipment. However, due to factors such as transportation conditions, differences in experimental equipment, etc., user test results may be inconsistent with factory data.

8. This kit has not been compared with similar kits from other manufacturers or products that detect the same target substance using different methods, so inconsistent test results cannot be ruled out.

9. The kit is for research use only. If it is used for clinical diagnosis or any other purpose, our company will not be responsible for any problems arising therefrom, nor will we assume any legal liability.

**Nanjing BYabscience technology Co.,Ltd**

**Website: www.byabscience.cn** **Official hotline: 025-5229-8998** **Supervision phone number:**